Novel method of minimal residual disease testing in myeloma: liquid biopsies to enumerate and 3D telomere-profile circulating tumor cells

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INTRODUCTION

In multiple myeloma (MM), the assessment of minimal residual disease (MRD) is a critical step in evaluating treatment efficacy in patients (Kumar et al., 2016). MRD assessment technologies that require invasive bone marrow (BM) aspiration do not allow for continuous monitoring, fail to account for MM's inherent variability (Gozzetti and Bocchia, 2023), and do not inform on the single MM cell biology. Three-dimensional (3D) telomere profiling, a measure of genomic instability, was shown to be a valuable prognostic biomarker in MM (Kumar et al, 2024). Evaluation of circulating tumor cells (CTCs) from peripheral blood (PB) with 3D-telomere profiling may provide a better insight into disease stability

AIM

We describe a new method for MRD evaluation that combines the enumeration and immunophenotyping of individual MM CTCs in liquid biopsy with 3D telomere profiling to characterize the residual MM cells or clones, and determine MRD negativity or positivity, enabling continuous non-invasive follow-up

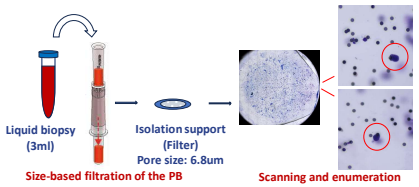
METHOD

Intact CTCs from the PB of 20 MM patients were isolated at different time points using size-based filtration with the ScreenCell® Cyto devices (ScreenCell, Paris, France).
CTCs were stained by modified Giemsa, imaged at 20x magnification, and enumerated. The malignant profile of the isolated CTCs was confirmed by 3D co-immuno-telomere fluorescent in situ hybridization (FISH) using human telomeres PNA probe and the CD56/CD138 antibodies.
3D telomere profiles of MM CTCs were analyzed by the TeloView® software.

RESULTS

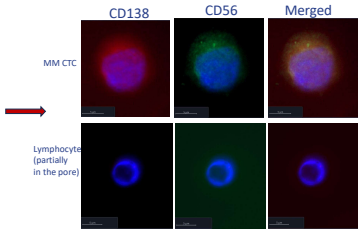
- The novel workflow successfully isolated, identified, and enumerated detectable CTCs not only at the time of diagnosis but also at various points in the disease course. CTCs were consistently isolated from all patient samples with high sensitivity (1 in 10^7). Malignant phenotype of the isolated CTCs was confirmed by IHC using the CD56/CD138 antibody panel.
- Hierarchical cluster analysis of the telomeric parameters of MM CTCs, measured by TeloView, identified distinct clusters. The canonical discriminant analysis showed strong group separation (Wilks' λ =0.00015, p < 0.0001), indicating that the cell features effectively distinguish the clusters based on the defined combinations of nuclear morphology and signal intensity patterns among the analyzed cells.

1. CTCs isolation and enumeration



Time point	Number of cases	Average CTC count/filter	Average total cell count/filter
At diagnosis	10	67.7	2.02E+07
Induction	6	10	
Relapse	4	33.6	

2. Immunophenotyping of isolated CTCs



Representative two-dimensional images of MM CTC and a normal lymphocyte labelled with CD138 and CD56 antibodies; cell nuclei are counterstained with DAPI

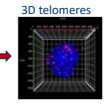
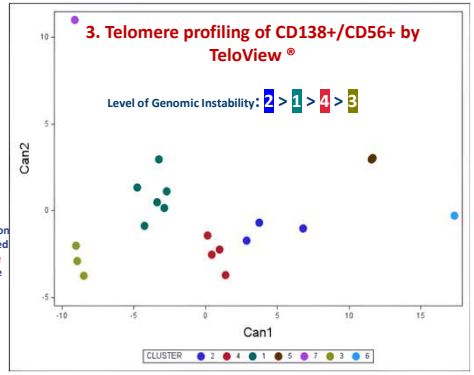


Figure 4: distribution of telomeres labelled with the PNA probe in the nuclear space (DAPI)

3. Telomere profiling of CD138+/CD56+ by TeloView ®



Canonical variables	
Can1	Can2
Larger nuclear volume	Overall signal intensity
Cells with signals farther from the nucleus center	Higher signal count and intensity.
Higher aggregate-to-cell ratio	Lower aggregate-to-cell ratio

CONCLUSIONS

Our novel workflow successfully identifies and enumerates detectable CTCs not only at the time of diagnosis but also at various points in the disease course. 3D telomere analysis of the isolated CTCs stratified the cohort into distinct clusters based on the 3D telomere profiles. This workflow enables longitudinal, minimally invasive monitoring of MRD in multiple myeloma patients without the need for a baseline sample, and yields functionally and biologically actionable data on CTCs. It enables a non-invasive assessment of disease stability or progression beyond simple enumeration. Further retrospective studies are planned to develop a TeloView-based risk-stratification model for MRD

REFERENCES

1. Gozzetti, A. and M. Bocchia. 2023. Liquid biopsy and blood-based minimal residual disease evaluation in multiple myeloma. Oncology Research *31*:271.

2. Kumar S, Rajkumar SV, Jevremovic D, et al. Three-dimensional telomere profiling predicts risk of progression in smoldering multiple myeloma. *Am J Hematol*. 2024;99(8):1532-1539.

3. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol*. 2016;17(8):e328-e346

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